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DIAGNOSING GVHD WITH A SIMPLE BLOOD TEST? CD4+ AND CD8+ T CELL GRANYZME B EXPRESSION MAY HOLD THE KEY

Srinivasan, S.[2], Hill, K.[1], Blazar, B.[3], Kean, L.[1] [1]Emory University; [2]Emory University; [3]University of Minnesota

The diagnosis of GvHD currently relies on clinical criteria coupled with histopathologic analysis of biopsy samples. However, the sickest patients are often too ill to permit GI or liver biopsy, resulting in significant diagnostic uncertainty for the patients who are at most risk for GvHD morbidity and mortality. Thus, there is a pressing need to develop a sensitive and specific test for GvHD that is amenable to routine monitoring in the peripheral blood.

Using the Primate GvHD Model we have determined that Granzyme B expression is massively upregulated in T cells causing GvHD. We analyzed the T cell phenotype in a cohort of Rhesus macaques who had received MHC-mismatched HSCT in the absence of immunosuppression, and who had histopathologically-proven GvHD. One of the most striking phenotypic changes that correlated with GvHD was the massive upregulation of Granzyme B on both CD4+ and CD8+ T cells: The per-cell Mean Fluorescence Intensity (MFI) for Granzyme B during GvHD increased by as much as 10-25-fold for both CD4+ and CD8+ T cells compared to pre-transplant expression levels.

Importantly, Granzyme B upregulation was independent of T cell proliferation: Animals that had undergone autologous transplant and who were undergoing T cell reconstitution by homeostatic proliferation did not upregulate Granzyme B on either CD4+ or CD8+ T cells. Thus, while the average Granzyme B MFI was $1.97x10^5$ and $1.15x10^5$ for CD8+ and CD4+ T cells causing GvHD, the MFI in CD4+ and CD8+ T cells expanding after autologous transplant was 9.6-fold and 11.7-fold lower, despite ongoing T cell proliferation in these animals ($p < 0.0001$). Immunosuppressed animals reactivating CMV also did not upregulate Granzyme B on either CD4+ or CD8+ T cells (Granzyme B MFI$0.24x10^5$ and $0.074x10^5$, respectively, corresponding to a 8.2-fold and 15.5-fold reduction compared to T cells causing GvHD, $p < 0.0001$).

Our results suggest that Granzyme B expression may be a highly discriminating phenotypic marker of GvHD, able to distinguish T cells causing GvHD from those undergoing homeostatic expansion or those activated during CMV viremia. This observation could be translated into a flow-cytometric test for GvHD in the peripheral blood, which could significantly improve our ability to accurately diagnose this devastating disorder. These observations also implicate Granzyme B in the pathogenesis of GvHD, and identify this enzyme as a potential target for GvHD prevention and treatment.

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GENETICALLY ENGINEERED DONOR T CELLS FOR BMT IMMUNOTHERAPY: EXPRESSION OF TRAIL AND PLZF SELECTIVELY ENHANCES GVT AND ABROGATES GVHD

Ghosh, A.[1], Holland, A.M.[1], Dogan, Y.[2], Yim, N.[1], Rao, U.K.[1], Young, L.F.[1], Smith, O.M.[1], Jenq, R.[1], Na, I.-K.[3], Tsai, J.J.[1], Singer, N.[1], West, M.[1], Penack, O.[3], Hanash, A.M.[1], Sauer, M.G.[4], Sant'Angelo, D.[5], Sadelain, M.[6], van den Brink, M.R.M.[1] [1]Memorial Sloan-Kettering Cancer Center, New York, NY; [2]Memorial Sloan-Kettering Cancer Center, New York, NY; [3]Charité CBF - Universitätsmedizin Berlin, Berlin, Germany; [4]Medizinische Hochschule Hannover, Hannover, Germany; [5]Memorial Sloan-Kettering Cancer Center, New York, NY; [6]Memorial Sloan-Kettering Cancer Center, New York, NY

We assessed two novel strategies in which T cells (mature and precursors) were genetically engineered to enhance select effector functions and improve graft versus tumor (GVT) effects without exacerbating graft versus host disease (GVHD).

One strategy relies on T cell cytolytic molecule TNF-Related Apoptosis Inducing Ligand (TRAIL), which induces apoptosis through death receptors (DR) 4 and 5 (only DR5 in mice). Certain tumor cells express high levels of DR5 making TRAIL an attractive candidate for engineering T cells to augment GVT. Mature T cells were transduced with a lentiviral TRAIL expression vector and adoptively transferred into lethally irradiated allogeneic bone marrow transplantation (allo-BMT) recipients, bearing LB27.4 tumors (B6 → CBF1+LB27.4). TRAIL+ T cells had enhanced antitumor effect compared to control-transduced T cells in vitro and upon transfer into tumor-bearing allo-BMT recipients ($p < 0.01$). Interestingly, the recipients of TRAIL+ T cells had significantly less GVHD. We generated TRAIL+ precursor (pre)T cells using the OP9-DL1 coculture system. Adoptive transfer of B6 TRAIL+ preT cells into syngeneic-transplanted BALB/c mice resulted in reconstitution with TRAIL+ T cells. When challenged with localized RENCA tumors, recipients of TRAIL+ preT cells had enhanced antitumor activity ($p < 0.05$) compared to recipients of control-transduced preT cells.

The second strategy explores the effect of overexpressing the transcription factor promyelocytic leukemia zinc finger (PLZF) in T cells. PLZF transgenic (tg) T cells have an effector/memory phenotype with innate-like characteristics. Following transfer, they cells display an altered cytokine profile with increased expression of TNFα and decreased IFNγ. Using adoptive transfer of CFSE-labeled donor T cells we observed that fast proliferating allo-responsive PLZF+ T cells died after only a few cell cycles. Using PLZF tg mice, we found that PLZF+ T cells cause less GVHD with intact GVT activity ($p < 0.001$) against A20 lymphoma. We then transduced T cells with PLZF expression vector and confirmed that donor PLZF+ T cells have less GVHD and intact GVT activity.

Our data suggest that adoptive therapy with genetically engineered TRAIL+ or PLZF+ T cells cause less GVHD while displaying intact or enhanced GVT activity. Furthermore, the "off the shelf" use of genetically enhanced preT cells represents a promising cell therapy strategy to enhance anti-tumor activity in both autologous and allo-BMT patients.

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CHANGES IN CD4$^+$ T CELLS AND REGULATORY T CELLS (TREG) IN CHRONIC GRAFT-VERSUS-HOST DISEASE (CGVHD)

Grogan, B.M.[1], Tabellini, L.[1], Storer, B.[1,2], Bumgarner, T.E.[1], Haeusermann, P.[1], Astigarraga, C.C.[1], Flowers, M.E.D.[1,2], Lee, S.J.[1,2], Warren, E.H.[1,2], Martin, P.J.[1,2], Hansen, J.A.[1,2] [1]Fred Hutchinson Cancer Research Center, Seattle, WA; [2]University of Washington, Seattle, WA

The role that CD4$^+$ T cells, specifically CD4$^+$FOXP3$^+$ Tregs, play in cGVHD following hematopoietic cell transplantation (HCT) is unclear. We performed a cross-sectional analysis of 218 HCT patients studied between 9 to 271 months post-HCT to test the hypothesis that increased activation and proliferation of CD4$^+$ T cells distinguishes patients with cGVHD from patients who have developed clinical tolerance (defined as no active chronic GVHD for 6 months without immunosuppression therapy {IST}), and to determine if changes in Treg are associated with cGVHD. Four groups were studied: patients with cGVHD on IST (n = 100), patients with cGVHD without IST (n = 66), tolerant patients (n = 52) and normal controls (n = 50). We observed no statistically significant difference in #Treg, %Treg/PBL or Foxp3 gene expression compared to cGVHD patients without IST, tolerant patients, and normal controls. Significant decreases in the # and %Treg, and Foxp3 gene expression, however, were present in patients with cGVHD on IST ($p = 0.004$, $p < 0.001$, and $p < 0.001$, respectively). We found decreased CD62L and increased CD95 expression on Treg in cGVHD patients without IST compared to normal controls (53.6±2.9% vs. 65.1±2.1%, $p = 0.001$, and 77.7±2.3% vs. 62.3±2.6, $p < 0.001$) which normalized in tolerant patients (65.4±1.8% p = ns, and 69.4±2.6%, p = ns). We observed increased activation, proliferation and apoptosis in the overall CD4$^+$ T cell population in cGVHD patients, especially without IST, with an increased expression of HLA-DR (5.8±0.6% vs. 3.5±0.3%, $p < 0.001$), CD95 (59.1±2.8% vs. 45.0±2.0%, $p < 0.001$) and Ki-67 (6.2±1.0% vs. 3.3±0.3%, $p = 0.008$) compared to normal controls. HLA-DR and Ki-67 expression normalized in tolerant patients (3.4±0.3%, p = ns; 3.9±0.5%, p = ns). The increased expression of activation markers in patients with cGVHD was accompanied by a decrease in the expression of CCR7 and CD62L, which normalized in tolerant patients. Proliferation and expression of activation markers were significantly decreased in cGVHD patients with IST compared to without IST. In summary, cGVHD is characterized by increased activation and proliferation of CD4$^+$ T cells compared to those from tolerant patients and healthy controls. The # and %Treg and Foxp3 expression were lower during IST but were not otherwise correlated with cGVHD. Treg in cGVHD show increased apoptosis and loss of receptors required for migration to peripheral tissues and lymph nodes, which may reduce Treg interactions with T effector cells.